Claims

It is claimed:

1. A method for determining the absolute quantity of a target biopolymer, such as a selected protein, in a crude solution, comprising the steps of:
   (a) adding a known quantity of an analog of said target biopolymer to said solution;
   (b) treating the target biopolymer and analog with a fragmenting activity to generate a plurality of corresponding biopolymer-fragment pairs;
   (c) resolving the biopolymer-fragment content of the mixture;
   (d) determining by mass spectrometric analysis the ratio of a selected target biopolymer to its corresponding analog; and
   (e) calculating, from said ratio and said known quantity of said analog, the quantity of the target biopolymer in the mixture.
2. The method of claim 1, wherein the biopolymer is selected from the group consisting of polypeptides and polynucleotides.
3. The method of claim 2, wherein the biopolymer is a polypeptide.
4. The method of claim 2, wherein the biopolymer is a polynucleotide.
5. The method of claim 1, wherein the solution is a crude fermenter solution, a cell-free culture fluid, a cell extract, or a mixture comprising the entire complement of proteins in a cell or tissue.
6. The method of claim 1, wherein either said target biopolymer or said analog is isotope labeled.
7. The method of claim 6, wherein said label is a stable isotope selected from the group consisting of ^{18}O, ^{15}N, ^{13}C, and ^{2}H.
8. The method of claim 7, wherein one of said target biopolymer and said analog is enriched in ^{15}N, and the other contains a natural abundance of N isotopes.
9. The method of claim 8, wherein said target biopolymer or said analog is produced synthetically using ^{15}N-enriched precursor molecules.
10. The method of claim 8, wherein the target biopolymer or analog enriched in ^{15}N is produced by a microorganism grown on ^{15}N-enriched media.
11. The method of claim 3, wherein said step of fragmenting is carried out by treating said solution containing said target polypeptide and said analog with a proteolytic enzyme.
12. The method of claim 11, wherein said proteolytic enzyme comprises trypsin.

13. The method of claim 1, wherein said step of resolving is effected by a chromatographic technique.

14. The method of claim 13, wherein said chromatographic technique is HPLC or reverse-phase chromatography.

15. The method of claim 1, wherein the target biopolymer is selected from the group consisting of enzymes, antibodies, receptors, hormones, growth factors, antigens, and ligands.

16. The method of claim 4, wherein said target polynucleotide is an oligonucleotide.

17. The method of claim 4, wherein said fragmenting step is carried out by treating said solution containing said target polynucleotide and said analog with a restriction enzyme.

18. The method of claim 17, wherein said restriction enzyme is a Type II restriction enzyme.

19. A method for verifying the presence and, optionally, determining the absolute quantity of a selected putative biopolymer in a mixture containing a plurality of isotope-labeled cellular biopolymer from a selected cell type; comprising the steps of:

(a) selecting a putative biopolymer potentially present in said mixture; generating a theoretical fragmentation of the putative biopolymer;

(b) generating a theoretical fragmentation of the putative biopolymer;

(c) selecting a theoretical fragment from the theoretical fragmentation;

(d) producing a biopolymer-fragment corresponding to said theoretical fragment;

(e) adding a known amount of the produced biopolymer-fragment as an internal standard to said mixture;

(f) treating said mixture with a fragmenting activity;

(g) resolving the cellular biopolymer-fragments along with the internal standard and analyzing the same by mass spectrometry to provide a mass spectrograph;

(h) locating a peak pair from said mass spectrograph comprised of a peak representing said internal standard and a peak representing a cellular biopolymer-fragment corresponding to said internal standard, thereby verifying the presence of said putative biopolymer;

(i) optionally, upon verifying the presence of said putative biopolymer, determining the ratio of internal standard to its corresponding cellular biopolymer-fragment; and,

(j) calculating, from said ratio and said known quantity of said internal standard, the absolute quantity of the putative biopolymer in the mixture.

20. The method of claim 19, wherein said putative biopolymer is derived from a database of sequence information.

21. The method of claim 19, wherein said putative biopolymer is selected from the group consisting of polypeptides and polynucleotides.

22. The method of claim 19, wherein said putative biopolymer is a polypeptide.

23. The method of claim 19, wherein said putative biopolymer is a polynucleotide.

24. The method of claim 19, wherein, in connection with said fragmentation step, the fragmentation of the cellular biopolymer is determined to be substantially complete with respect to the cellular biopolymer fragment corresponding to said internal standard.

25. The method of claim 22, wherein the fragmentation step is carried out by treating said solution containing said target polypeptide and said analog with a protease.

26. The method of claim 23, wherein the fragmentation step is carried out by treating said solution containing said target polynucleotide and said analog with a restriction enzyme.

27. The method of claim 19, further comprising:

(k) after determining the absolute quantity of the putative polypeptide in the mixture, growing the selected cell type under a set of defined conditions,

(l) querying an extract from the grown cell type for the presence, for an increase or decrease of the absolute concentration of said putative polypeptide by mixing the extract with a known amount of the isotope-labeled mixture as a new internal standard;

(m) treating the extract with a proteolytic activity;

(n) resolving the polypeptide fragment content of the extract and analyzing the same by mass spectrometry to provide a mass spectrograph;

(o) locating a peak pair from said mass spectrograph comprised of a peak representing said new internal standard and a peak representing a cellular polypeptide fragment corresponding to said new internal standard, thereby verifying the presence of said putative polypeptide;

(p) optionally, upon verifying the presence of said putative polypeptide, determining the ratio of the new internal standard to its corresponding cellular polypeptide fragment; and,

(q) calculating, from said ratio and said known quantity of said internal standard, the absolute quantity of the putative polypeptide in the extract.

28. A cell-culture extract, derived from a selected microorganism grown on media enriched in a specific isotope, said extract containing a known amount of a metabolically labeled biopolymer determined by a biopolymer-separation technique in combination with mass spectroscopy.

29. A method for determining the identity of a target biopolymer fragment in a solution, comprising the steps of:

(a) adding an analog of said target biopolymer and said target biopolymer to said solution, in a selected analog:target ratio;

(b) treating the target biopolymer and analog with a fragmenting activity to generate a plurality of corresponding biopolymer-fragment pairs;

(c) resolving the biopolymer-fragment content of the solution;

(d) identifying by mass spectrometric analysis those biopolymer-fragment pairs that exhibit the selected ratio; and, optionally,

(e) determining the biopolymer sequence of the biopolymer-fragment pairs identified in step (d).

30. The method of claim 29, wherein said target biopolymer is a protein.

31. The method of claim 29, wherein said target biopolymer is a polynucleotide.

32. The method of claim 29, wherein said crude solution contains a plurality of different biopolymers.

33. The method of claim 32, wherein the solution is a crude fermenter solution, a cell-free culture fluid, a cell extract, or a mixture comprising the entire complement of biopolymers in a cell or tissue.